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# Muscle distribution of sylvatic and domestic *Trichinella* larvae in production animals and wildlife

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**Abstract**

Only a few studies have compared the muscle distribution of the different *Trichinella* genotypes. In this study, data were obtained from a series of experimental infections in pigs, wild boars, foxes and horses, with the aim of evaluating the predilection sites of nine well-defined genotypes of *Trichinella*. Necropsy was performed at 5, 10, 20 and 40 weeks post inoculation. From all host species, corresponding muscles/muscle groups were examined by artificial digestion. In foxes where all *Trichinella* species established in high numbers, the encapsulating species were found primarily in the tongue, extremities and diaphragm, whereas the non-encapsulating species were found primarily in the diaphragm. In pigs and wild boars, only *Trichinella spiralis*, *Trichinella pseudospiralis* and *Trichinella nelsoni* showed extended persistency of muscle larvae (ML), but for all genotypes the tongue and the diaphragm were found to be predilection sites. This tendency was most obvious in light infections. In the horses, *T. spiralis*, *Trichinella britovi*, and *T. pseudospiralis* all established at high levels with predilection sites in the tongue, the masseter and the diaphragm. For all host species, high ML burdens appeared to be more evenly distributed with less obvious predilection than in light infections; predilection site muscles harbored a relatively higher percent of the larval burden in light infections than in heavy infections. This probably reflects increasing occupation of available muscle fibers as larger numbers of worms accumulate. Predilection sites appear to be influenced primarily by host species and secondarily by the age and level of infection.


*Keywords:* *Trichinella* spp.; Predilection; Muscle distribution; Pigs; Wild boars; Horses; Foxes

## 1. Introduction

The muscle distribution of the different *Trichinella* genotypes in a given host is not only important for meat inspection of production animals, where sylvatic *Trichinella* species are occasionally found, but also for epidemiological surveillance in indicator animals, e.g. foxes and wild boars. In this study, muscle tissue was obtained from a series of experimental infections in pigs, wild boars, foxes and horses, with the aim of evaluating the predilection sites of 9 well-defined genotypes of *Trichinella*.

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## 2. Materials and methods

### 2.1. Parasitological material

Nine genotypes of *Trichinella*, all registered at the International *Trichinella* Reference Centre (TRC) in Rome, were used for inoculations: *Trichinella spiralis* (T1) (ISS004, Maryland, USA, domestic pig), *Trichinella nativa* (T2) (ISS042, Alaska, *Ursus maritimus*), *Trichinella britovi* (T3) (ISS100, Italy, *Canis lupus*), *Trichinella pseudospiralis* (T4, USSR) (ISS013, Caucasus, USSR, *Procyon lotor*), *T. pseudospiralis* (T4, USA) (ISS470, Alabama, USA, *Coragyps atratus*), *T. pseudospiralis* (T4, AUST) (ISS141, Australia, *Dasyurops maculatus*), *Trichinella murrelli* (T5) (ISS035, Pennsylvania, USA, *Ursus americanus*), *Trichinella* sp. (T6) (ISS034, Montana, USA, *Ursus arctos*), *Trichinella nelsoni* (T7) (ISS037, Tanzania, Africa, *Phacochoerus aethiopicus*). The parasites were propagated by serial passage in outbred Ssc:CF1 mice. Muscle larvae (ML) used for the inoculation of pigs were recovered by digestion and sedimentation as described below.

### 2.2. Experimental animals

Muscle samples were obtained from a total of 102 pigs, 36 wild boars, 30 horses and 108 foxes and necropsy was performed at 5, 10, 20, and 40 weeks post inoculation. All animals were treated in accordance with the animal ethics laws of Denmark.

### 2.3. Larval recovery and tissue digestions

From all host species, the number of ML was determined in corresponding muscles/muscle groups by artificial digestion according to Kapel and Gamble (2000). The muscles sampled were: the diaphragm (lumbar and costal parts), the tip and base of tongue (m. lingua anterior/posterior), the lower jaw (m. masseter), the abdomen (m. rectus abdominus), the tenderloin (m. psoas minor), the neck (m. splenius), the shoulder (m trapezius), the throat (m. sternohyoideus), the upper forelimb (m. biceps-/m. triceps brachii), the lower forelimb (flexor digitorum), the upper hindlimb (m. quadriceps), the lower hindlimb (m. gastrocnemius), the upper jaw (m. temporalis), the intercostals (m. intercostales), the rump (m. gluteus maximus/medius), the filet (m. longissimus dorsi). To compare predilection sites in animals with high and low infection levels, a score (*I*%) indicating the relative larval burden was calculated for each muscle group, using the highest number of larvae per gram (lpg) in each animal as the 100% reference point (Table 1). This value was used for statistical comparison of particular host–parasite combinations and finally to rank muscle larvae intensity in the examined muscles.

A rough estimate of digestibility of the different muscle tissues was performed on 20 g samples of surplus tissues (except for fox tongue where there was no extra tissue). For this, each tissue sample was minced into 3 mm pieces in a grinder and digested individually at 45 °C in 500 ml digestion fluid (500 ml $H_2O$, 5 ml HCl, 5 g pepsin (1:10.000 NF)) in a 1000 ml beaker on a heated magnetic stirrer with digital reading of temperature in the fluid. At 30 min intervals, the fluid was poured though a 200 μm sieve into another pre-heated beaker (45 °C) and the amount of any retained tissue was determined. If more than 0.2 g tissue was retained, the tissue was returned into the digestion fluid for another 30 min. Complete digestion was defined as having less than 0.2 g of tissue retained on the sieve.

### 2.4. Statistical analysis

The rank of relative larval burden (*I*%) of muscle tissues in different host parasite combinations was compared by a Friedman two-factor analysis of variance (Campbell, 1974). Comparisons were done according to host species, age of infection, infection level, and encapsulating versus non-encapsulating species.

## 3. Results

When comparing the ranking of infection levels in different muscles for particular host parasite combinations, no significant differences were found for the respective *Trichinella* species in pigs, wild boars, or horses (Table 1). In contrast, there was a significant difference in the tissue distribution of encapsulating and non-encapsulating species in foxes. No statistical

Table 1
Rank of predilection sites of encapsulating and non-encapsulating *Trichinella* spp in muscle tissues of experimentally infected animals

| | Pig/wild boar | | Horses | | Foxes | |
|---|---|---|---|---|---|---|
| | *T. spiralis*, *T. nativa*, *T. britovi*, *T. nelsoni* | *T. pseudo* (Russia) (Australia) (USA) | *T. spiralis*, *T. britovi* | *T. pseudo* (Russia) | *T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli*, *Trichinella* T6 | *T. pseudo* (Russia) (Australia) (USA) |
| Tongue base | 1 | 2 | 1 | 3 | 1 | 9 |
| Diaphragm | 2 | 1 | 3 | 1 | 3 | 1 |
| Masseter | 6 | 3 | 2 | 2 | 6 | 7 |
| Tongue tip | 3 | 5 | 4 | 4 | | |
| Neck | 4 | 4 | 5 | 5 | 7 | 5 |
| Abdomen | 7 | 8 | 6 | 8 | | |
| Tenderloin | 10 | 7 | 7 | 6 | | |
| Throat | 5 | 6 | | | | |
| Shoulder | | | 9 | 7 | | |
| Intercostals | 9 | 10 | 10 | 11 | | |
| Upper jaw | 11 | 12 | | | | |
| Upper forelimb | 13 | 9 | 11 | 9 | 4 | 2 |
| Lower forelimb | 14 | 13 | 8 | 14 | 2 | 8 |
| Upper hindlimb | | | 14 | 10 | 5 | 4 |
| Lower hindlimb | 12 | 11 | 12 | 15 | 8 | 6 |
| Rump | 8 | 14 | 13 | 13 | | |
| Filet | 15 | 15 | 15 | 12 | 9 | 3 |

2 h digestion of 10–100 g minced (3 mm) tissue sample in 500 ml HCl/pepsin by magnetic stirrer technique at 45 °C. Rank: 1: highest mean infestation; blanks: No muscles sampled.

differences in ML distribution were found based on the age of infection.

In foxes, where all *Trichinella* species established in high numbers, the encapsulating species were found in highest densities in the tongue, extremities and diaphragm, whereas the non-encapsulating species had a predilection site in the diaphragm. In pigs and wild boars, only *T. spiralis*, *T. pseudospiralis* and *T. nelsoni* showed persistency of ML, but for all genotypes the tongue and the diaphragm were found to be predilection sites. The tendency for predilection in these sites was most pronounced in light infections. In the horses, *T. spiralis*, *T. britovi*, and *T. pseudospiralis* all established at high levels, with predilection sites in the tongue, the masseter and the diaphragm.

Table 2
Digestion time (hours) of muscle tissue from different hosts

| | Pig/wild boar | Foxes | Horses |
|---|---|---|---|
| Tenderloin | 1/2 | | 1/2 |
| Filet | 1/2 | 1/2 | 1/2 |
| Diaphragm | 1/2 | 1/2 | 1/2 |
| Rump | 1 | | 1/2 |
| Upper hindlimb | | 1 1/2 | 1 |
| Intercostals | 1 | | 1 |
| Neck | 1 | 1 1/2 | 1 |
| Masseter | 1 | 1 1/2 | 1 |
| Shoulder | | | 1 |
| Abdomen | 1 | | 1 |
| Upper forelimb | 1 | 1 1/2 | 1 |
| Lower hindlimb | 1 1/2 | 1 1/2 | 1 |
| Lower forelimb | 1 1/2 | 2 | 1 1/2 |
| Tongue base | 1 1/2 | 2 | 1 1/2 |
| Tongue tip | 1 1/2 | | 1 1/2 |

Complete digestion of 20 g minced (3 mm) tissue sample in 500 ml HCl/pepsin by magnetic stirrer technique at 45 °C.

Digestibility of muscles varied greatly as illustrated in Table 2, with the tenderloin, filet and diaphragm being digested within 1/2 h, and the tongue and the lower parts of the legs requiring 1 1/2–2 h for complete digestion. For corresponding muscle groups, the fox tissue took longer to digest than either pork or horse meat.

## 4. Discussion

Although the predilection of *T. spiralis* in pigs has been extensively studied (Zimmermann, 1970; Kotula

et al., 1984), the few studies comparing different species of *Trichinella* in pigs or wild boars (Pozio et al., 1985; Ooi et al., 1994; Kapel et al., 1998; Serrano et al., 1999; Kapel, 2001) are supported by the findings of the present study in that all *Trichinella* species preferentially accumulate in the diaphragm and the tongue of wild boars and pigs. For horses, our studies with *T. spiralis* are in accordance with research previously presented (Smith and Snowdon, 1987; Polidori et al., 1989; Soule et al., 1989a,b; Gamble et al., 1996; Voigt et al., 1997; Pozio et al., 1999) indicating that predilection sites include the diaphragm, the masseter and the tongue. Interestingly, comparable predilection sites have also been found in other herbivores (Smith and Snowdon, 1989; Alkarmi et al., 1990; Pajersky et al., 1996a,b; Theodoropoulos et al., 2000). The predilection sites of encapsulating *Trichinella* species in foxes (lower forelimb, the tongue and the diaphragm) are comparable to those found for *T. spiralis* in red foxes (Noeckler and Voigt, 1997) and *T. nativa* in arctic foxes (Kapel et al., 1994, 1995). Interestingly, the predilection sites found for the non-encapsulating *T. pseudospiralis* differ, where the diaphragm, the upper forelimb and the filet are the preferred sites of infection. Although not significant for pigs and horses, non-encapsulating species generally showed a predilection for the diaphragm and the encapsulating species showed a predilection for the tongue.

For all host species, high ML burdens showed a more even tissue distribution when compared with light infections; predilection site muscles harbored a relatively higher percent of the larval burden in light infections than in heavy infections. This most likely reflects an increasing occupation of available muscle fibers in heavier infections, but since the experimental infections in this study were not conducted with different doses for the individual host–parasite combinations the results did not allow for any statistical evaluation. Predilection sites were primarily influenced by host species and secondly by the age and level of infection. Taking the digestibility of the respective muscle groups into account, the diaphragm appears to be the muscle of choice for inspection in pigs and horses as the masseter in horses takes longer to digest. Considering differences in the distribution of non-encapsulating and encapsulating species, the tongue offers an easily assessable and distinguishable muscle for use in wildlife surveillance; however, the long digestion time for this muscle should also be considered.

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